LINKS/MS2LINKS FAQs

RUN TIMES

Q: Why do I have very long run times? Is there a way to tell if the program is still running or already hang?

A: The vast majority of users jobs still take just a few seconds to a couple of minutes actual compute time. The long run time is due to the complexity of the problem. The algorithms used in Links are exponential, meaning adding just a little bit more complexity greatly increases compute time, or inversely, efforts to simplify the problem a little bit may give very drastic increases in response time. There is no set time limit, but the server goes down for backups every Saturday so any running jobs are killed then.

Ways to significantly reduce the number of combinations processed include:

1. Fixing the ends of the crosslinker:
   Each XLINK sequence position that you can fix rather than leave as '*' ought drop total combinations by a couple orders of magnitude.

2. Reducing the total number of allowed modifications for a MOD ought to halve the number of combinations. Using an '*' defaults to 3 applied modifications), so, putting in '2' or '1' instead of '*' for modifications will help.

3. Reducing the number of missed cleavages for the XASEs will also help, but the amount it helps is very dependent on where the XASE cleaves (anywhere from very little difference to orders of magnitude fewer combinations)

Q: Will links still run even if I log out of the portal?

A: Yes. You can check the Data Browser for output files to check when they are done.

Q: Is there a way to abort a session?

A: At the moment, there is no way of aborting a session.

RUN ERRORS

Q: Links crashed in the middle of a job and gave me the following error message:

    ; nested exception is: javax.xml.rpc.JAXRPCException
    All available files have been saved to the resource directory
under 'Links/Results//Links'.

A: If you see this error message please contact the CMS3D team. This error is portal-related.

Q: I got this error while running Links:
    Job pool full error

A: The web service that runs Links and Ms2links is set to handle a couple of running jobs at the same time, so if there are a lot of active jobs, it intentionally returns this message. Jobs are queued so you should be able to check later for the status of your runs.

Q: I tried to run links today and the first time there was an error--are the error reports filed somewhere?

A: Yes. Errors associated with running Links and MS2Links are compiled in the jqe.err file that is generated together with the other output files.

Q: have posted the following error message at the Chat Room:

"Links Excecution Failed Links has failed for the following reasons. ; nested exception is: java.net.ConnectException: Connection refused All avaliable files have been saved to the resource directory under 'Links/Results//mass883p8460_2' ".

A: The Links webservice was intermittently unavailable between due to work on upgrading the production portal. The C-MS3D admin sends out announcements when the portal will be down for service.

FILE NAMING

Q: I wasn't able to download or open my results. My files are named 2-22-#67-1 and so on.

A: You may want to rename your files and avoid putting a # sign in the name.

DEFINING MODIFICATIONS

Q: Links doesn't take into account exceptions to trypsin's cleavage rules (cleaves after K,R except if followed by a proline on the C-term end).

A: We use a special syntax for exceptions. The syntax to handle exceptions is the " ^ " (caret) symbol. To define trypsin cleavage rules with the exception (cleaves after K,R except if followed by a P on the C-term end):

     XASE   1   **RK|*^P** 2   0   0   Tryp

Q: How many conditions can be run in a single session? For example, and what is the maximum number of modification fields that can be run in addition to at least two cross-linker fields? For that matter, how many cross-linking fields can be accommodated by Links?

A: For the modification table, I believe we have set a max of 20 definitions total. However, be cautioned about the complexity and run times associated with such jobs (please see comment above)

CONTROL PEAKS

Q: When subtracting sample peaks with control peaks, is the specificity linked to the error threshold in the parameters file?

A: No. The program actually looks for exact duplicates of peaks.

MS2LINKS

Q: I have been trying to use MSlinks for my crosslinking data and I am a little confused with the output file. First of all, the excel file (the output comes with) does not show the corresponding matches for the peak list I enter at the beginning. What I get is the list of all possible calculations for the mass of fragmented crosslinked peptides. It does not give simple b or y ions or internal fragments. I tried to test the software by entering only the simple b and y ions from either peptide without the crosslinked region and it does not give a prediction for them. Before it used to calculate all

possibilities and output the results with their differences from the expected theoretical mass. Can you please enlighten me with this problem with the output files.

A: Please go back to your parameter file definitions. You may have opted to write out the theoretical library instead. This is the write_library option.

Q:In MS2 links, does the program have the ability to take a full sequence, predict all peptides based on protease, subsequently predict dipeptides resulting from cross-linking, and then compare to MS/MS data provided in the peaklist? I have tried to do this test experiment, but am not getting the expected results.

A: MS2Links is capable of handling top-down sequencing experiments of full protein sequences. However, it cannot perform the specific task that you are describing. For this, you need to use Links first to match the crosslinked peptides resulting from protease digestion. And once you have a putative match + MS/MS data, then you need to switch to MS2Links.

Q: Do the peptides need to be included in the database separately as individual entries?

A: Yes, the peptides need to be added as separate "sequences" in the fasta file for MS2LINKS and you can just define the pairs in the mod table. You can input up to 30 sequences and consolidate all your MS/MS peaklists in 1 peaklist file if you are dealing with the same crosslinker, so you can run the program once.